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Mechanistic approach to phytoremediation of water

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Abstract

Conventional thinking regarding the use of food crops to clean aquaculture effluents has been that plants cannot remove nutrients in water to low levels without a reduction in productivity and quality. Because greenhouse space is expensive, productivity is critical for a profitable operation. A production strategy, called the conveyor production system (CPS), was developed using thin-film technology for plant production in dilute aquaculture effluents. With the CPS, young plants were positioned near the solution inlet in a gutter receiving the effluent and moved progressively, like along a conveyor belt, towards the outlet as they grew. Luxury consumption by lettuce plants (*Lactuca sativa* L. cv. Ostinata) enabled them to store P in their tissues early in their growth cycle for use later as water P levels decreased and influx could no longer meet current demands. If water is distributed in a horizontal plug-flow pattern, without the CPS, all nutrients will be luxury consumed at the inlet, making nutrients limiting at the outlet and significant greenhouse space will be dedicated to growing plants that have no market value. The object of this study was to construct and operate a pilot-scale CPS, collect data demonstrating its potential to clean effluent and produce a marketable product, and develop a mechanistic model describing the process. Greenhouse studies demonstrated that by using the CPS, phosphorus could be reduced from 0.52 to <0.01 mg l^{-1} by lettuce without an apparent reduction in production or quality. The mechanistic model described in this paper simulated experimental data collected during the operation of the CPS growing lettuce and defines critical data necessary for the general comparison of effluents for treatment.
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Keywords: Phosphorus; Nitrogen; Biological nutrient removal; Hydroponics; Effluent; Phytoremediation; Wastewater

1. Introduction

The decline of federal subsidies for municipal wastewater treatment in the United States is driving the search for lower cost alternatives (Jewell, 1991, 1994). Confounding this quest for

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lower cost systems, however, is the move towards more stringent nutrient discharge regulations. There are a number of biological, chemical, and physical methods to remove P from wastewater (Metcalf and Eddy Inc., 1991), some of which are capable of removing it to very low levels. However, treatment costs increase substantially as the required discharge concentration decreases (Adler et al., 2000a). This environment has led to increased interest in natural systems for wastewater treatment (Reed et al., 1995), and the incorporation of ecological principles to increase sustainability and balance production goals with their impact on the environment (Costanza et al., 1997; Bergen et al., 2001). One way to reduce water treatment costs is to produce a product of value concomitant with treatment of the water (Adler et al., 1996b,c, 2000a,b). Higher plants have been a critical component in ecologically engineered systems (Todd and Josephson, 1996). They have also been used to remove heavy metals from water by a process called rhizofiltration (Dushenkov et al., 1995). Developing a mechanistic understanding and design criteria for these systems to maximize their potential is important.

Aquacultural effluents are difficult to treat because they contain relatively dilute nutrients, <1 mg l^{-1} P (Heinen et al., 1996). Because aquacultural facilities generate large volumes of this dilute effluent, the quantity of nutrients discharged to receiving waters can be large. Effluent treatment is important because mass loading is the critical factor contributing to environmental degradation by nutrients.

The increased competition for limited water resources among urban, industrial, and agricultural interests has lead to increased use of reclaimed wastewater for irrigation (National Research Council, 1996), especially in arid locations. Aquaculture effluents contain relatively low quantities of P, about 5–10% that contained in reclaimed wastewater. Land application of wastewater can significantly reduce treatment costs compared with standard water treatment technologies (Adler et al., 2000a). However, there still are significant costs associated with treatment and in Adler et al. (2000a), only production of a higher value product using thin-film technology had the potential to generate a profit. Thin-film technology, described in this paper, allows plants to selectively extract nutrients from water making dilute effluents an equivalent source of nutrients as more concentrated effluents.

Predominant thinking regarding the use of food crops to clean aquaculture effluents has been that plants cannot remove nutrients in water to low levels without a reduction in productivity and quality. Because greenhouse space is expensive, maintaining maximum productivity is critical to sustaining a profitable operation.

The object of this study was to construct and operate a pilot-scale conveyor production system (CPS) using thin-film technology to grow lettuce plants (*Lactuca sativa* L. cv. Ostinata) on rainbow trout effluent (RTE), collect data demonstrating its potential to clean effluent and produce a marketable product, and develop a mechanistic model describing the process.

1.1. Thin-film technology production system

Thin-film technology is a hydroponic crop production system in which plants grow in water that flows continuously as a thin-film over their roots. Water flow across the roots decreases the stagnant boundary layer surrounding each root. This enhances the mass transfer of nutrients to the root surface and permits crops to maintain high productivity at steady-state phosphorus levels above 0.3 mg l^{-1} (Asher and Loneragan, 1967; Chen et al., 1997).

Conventional hydroponic production of lettuce and basil using thin-film technology, also known as Nutrient Film Technique (NFT), was investigated as a method to remove P from an aquaculture effluent. In our initial study, lettuce plants were grown in long (21.9 m, 126 plants) troughs on RTE flowing from one end of the trough to the other. This system removed P from an inlet concentration of $\approx$0.7 mg l^{-1} to an outlet concentration of a few μg l^{-1}. However, as solution P concentrations dropped below $\approx$0.3 mg l^{-1}, tissue P concentrations decreased. Even so, growth was sustained until the P concentration within the plant dropped below the critical deficiency level (CDL, 0.35–0.4% P on a dry weight basis for lettuce). At that point, P deficiency symptoms appeared, growth rate decreased, and the plants became unmarketable. Thus, conventional hydroponic technology (where all plants in the trough are the same age) could only remove ≈ 50% of the P while producing a marketable product. Although lettuce can remove P to <0.3 mg l^{-1}, a reduction in growth coincides with a further reduction in solution P concentrations. As a result, the CPS was developed to sustain plant productivity and health while removing dissolved P levels to less than 0.01 mg l^{-1}.

1.2. Conveyor production system

Plants have the capacity to absorb and store nutrients in excess of their immediate needs, a process called luxury consumption (Marschner, 1995). The CPS is a production strategy which uses thin-film technology (Adler et al., 2000a), but enables plants to store P early in their growth cycle. This stored reservoir of P can be remobilized to meet current plant needs and supplement the lower P influx rate, which occurs as P drops below ≈0.3 mg l^{-1} in the effluent. Phosphorus remobilization will maintain growth as long as the tissue P concentration remains above the CDL. At the front end of the thin-film troughs, where nutrient concentrations are highest, young plants absorb and store nutrients in excess of their immediate needs. Luxury consumption of nutrients during this early growth phase sustains the plants when they are moved towards the trough outlet, where nutrient concentrations in solution are too low for absorption kinetics to meet their growth needs. Cellular nutrient concentrations are sufficient to sustain growth even after the concentrations of nutrients in the water became limiting. The CPS permitted the removal of P in effluents to <0.01 mg l^{-1} while concurrently producing a high-value crop without an apparent reduction in yield or quality. This contrasts with a conventional production system in which a gradient in growth and a reduction in plant quality would accompany the reduction in nutrient levels.

1.3. Mechanistic model

Mechanistic models have been developed to describe nutrient uptake by plant roots growing in soil. Barber (1995) used a mechanistic approach to evaluate soil nutrient bioavailability. This has reduced the need to conduct numerous crop–fertilization experiments to gain information about each crop–soil combination, and it has increased our understanding of the soil nutrient-availability process. Several studies have been conducted using plants to remove nutrients from wastewater (Berry et al., 1980; Rakocy and Hargreaves, 1994; Adler et al., 2000a). However, since nutrient removal from wastewater depends on the concentration, plants species, and environmental conditions, application of site specific data to other locations is of limited value. This paper takes a mechanistic approach in the use of plants to remove nutrients from wastewater similar to that which Barber (1995) used successfully to model nutrient uptake in soil systems. A mechanistic approach is presented because it provides a framework to direct research and identifies data that needs to be collected so experiments based on different effluent sources can have more general application.

2. Methods

This section describes the methods used to collect data during operation of the pilot-scale CPS (Section 2.1) and how the parameters for the model were collected (Section 2.2), followed by a description of the model (Section 2.3).

2.1. Conveyor production system

The CPS (Fig. 1) consisted of six connected gutters, roughly 10.2 × 366 cm (Genova Products, Inc., Davison, MI). The gutters were covered with 1.6 mm PVC and had 3.2 cm holes evenly spaced at 17.4 cm intervals, and were planted with 20 seedlings. Plants were grown from the beginning of June through mid-July in a greenhouse kept at 27–34 °C during the day and at 21–24 °C during the night. With this production strategy, the rate of biomass production per unit area, hydraulic load-

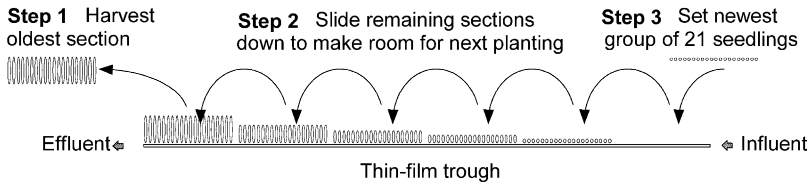

Fig. 1. Schematic of the CPS.

ing rate, and effluent phosphorus concentrations were relatively constant. Lettuce plants were harvested every 4 days, so lettuce was in the system for 24 days. Plant rotation was as follows: plants were harvested at the outlet end of the system, the plants in the remaining five sections were moved down one position, and 16-day-old lettuce plants were set into the system at the inlet end (Fig. 1). This cycle was repeated six times to move a given set of plants completely through the system to harvest.

2.1.1. Growth conditions

'Ostinata' lettuce (a butterhead-type lettuce) were seeded into Oasis® cubes (LC-1 Horticubes, Smithers-Oasis, Kent, OH) and placed in a darkened growth chamber for 24 h at 20 °C. By the end of the first week, seedlings had been selected for uniformity and thinned to one plant per cube. When 8-days-old, lettuce seedlings were moved from shallow trays to recirculating thin-film gutters; they were watered for the first 16 days with a complete nutrient solution (in mM): 3 $Ca(NO_3)_2$, 4 KNO_3, 1 KH_2PO_4, and 2 $MgSO_4$. The solution also contained the following micronutrients (mg l^{-1}): Fe as $FeSO_4$ (2.5) and DTPA (2.5), B as H_3BO_3 (0.5), Mn as $MnSO_4$ (1.0), Zn as $ZnSO_4$ (0.05), Cu as $CuSO_4$ (0.02), and Mo as $(NH_4)_6Mo_7O_{24}$ (0.01). The solution was adjusted to pH 6.0 with KOH. At 16-days-old, the lettuce seedlings were moved to either non-recirculating thin-film systems with individual gutters for determination of model parameters or a series of six gutters configured with the CPS.

Seedlings were initially grown in a concentrated nutrient solution because the dilute solution did not deliver enough nutrients to the root surface to satisfy demands. The only nutrients delivered to the root surface before they emerge from the cube are due to evapotranspiration or mass flow, because diffusion distances are too far. Seedlings could grow on effluent from the beginning if a hole in the Oasis cubes was extended down through the bottom to permit immediate entry of roots into the effluent upon germination. Without the hole, plants became nutrient starved because mass transfer of nutrients was poor if the roots were not in the solution. The concentration of nutrients in solution would have to be up to 10–30 times greater than that contained in the effluent to be sufficient to meet lettuce needs through mass flow alone. At an evapotranspiration rate of 0.25 l g^{-1} dry wt., a tissue concentration of 4.5% N, 0.5% P, and 6.5% K, would require solution concentrations of 180 N, 20 P, and 260 K mg l^{-1}, to be balanced for N, P, K, and water.

2.1.2. Rainbow trout effluent characteristics

The RTE to be treated was from the recirculating system for rainbow trout production at The Conservation Fund's Freshwater Institute, Shepherdstown, WV. The bulk RTE typically has a pH of 7.2 and contained around 6 mg l^{-1} total suspended solids (TSS) and the following macronutrients (mg l^{-1}): NO_3-N (25), P (0.52), K (5), Ca (55), Mg (20), and S (9). In this effluent, nutrients most limiting to the plants were Fe, Mn, and then K. A plant's productivity is determined by the nutrient present in lowest amount, relative to its requirements. When other nutrients limit plant growth, phosphorus removal can be increased by adding the nutrients that are most limiting. To maximize phosphorus removal, the following nutrients were added to make phosphorus the most limiting nutrient: 0.1 mg l^{-1} Fe–EDDHA (LibFer SP, Allied Colloids Inc., Suffolk, VA), 0.1 mg l^{-1} Mn–EDTA (Librel Mn, Allied Colloids Inc.), 0.004 mg l^{-1} Mo (as $(NH_4)_6Mo_7O_{24}$, and 15 mg

l^{-1} K (as K_2SO_4). RTE was pumped with peristaltic pumps (model no. 7520-35 Cole Parmer Instrument Co., Chicago, IL) at a constant flow rate of 300 ml min^{-1} (hydraulic loading rate about 0.08 cm min^{-1}) for lettuce in the CPS.

2.1.3. Ion analysis

In water samples, NO_3-N and P were quantified by ion chromatography (APHA, 1998 4110.B). A Dionex Series 4500i ion chromatograph (Sunnyvale, CA), was equipped with a gradient pump and conductivity detector for use in anion analysis. A Dionex IonPac-AG4A guard plus AS4A separator column was followed by an anion micromembrane suppresser to reduce background conductivity. The eluent was 1.80 mM Na_2CO_3 and 1.70 mM $NaHCO_3$ at a flow rate of 2.0 ml min^{-1}. The regenerant for the suppresser was 25 mN H_2SO_4 at a flow rate of 3.0 ml min^{-1}. The sample loop volume was 2.0 ml. A three-point standard curve was developed which bracketed the concentration of the samples that were analyzed. With this system, both NO_3-N and P were linear over a broad range (0.1–100 mg l^{-1} for NO_3-N and 0.001–1.0 mg l^{-1} for P). Potassium was determined by atomic emission spectroscopy (Perkin–Elmer 4000, Norwalk, CT).

In tissue samples, total N was determined with a FP-428 LECO nitrogen determinator (AOAC, 2002 990.03) (LECO Corporation, St. Joseph, MI). For analysis of total P, a 0.1 g (dry wt.) sample was ground and weighed into graduated 50 ml Kimax Folin-Wu digestion (N.P.N.) tubes, digested with perchloric acid, and quantified by ion chromatography as described by Adler (1995). Potassium was determined by atomic emission spectroscopy (Perkin–Elmer 4000).

2.2. Model parameter measurements

Plants for model parameters were grown in four replicated gutters from mid-April through the end of May in a greenhouse kept at 24–28 °C during the day and at 16–18 °C during the night. The day was extended with 8 h of supplemental light from high-pressure sodium lamps (130 µmol m^{-2} s^{-1} PPF).

A synthetic rainbow trout effluent (SRTE) was used in studies to derive model parameters to obtain a consistent effluent and eliminate complicating effects caused by solids. The SRTE was made by adding nutrients to spring water which typically contains (mg l^{-1}): NO_3 (3), P (< 0.001), and K (3). In the actual RTE, P and K are co-limiting after Fe, Mn, and Mo is added. Therefore, influx kinetics was determined for P and K, P with the P-limited SRTE and K with the K-limited SRTE. The following nutrients were added to the spring water for the P-limited SRTE (mg l^{-1}): NO_3 was added as HNO_3 and KNO_3 (12), P as H_3PO_4 (0.7), K as KNO_3 (17). The K-limited SRTE was made up as follows (mg l^{-1}): NO_3 was added as HNO_3 and KNO_3 (12), P as H_3PO_4 (2.0), K as KNO_3 (5). Micronutrients were added in the same quantities for both P- and K-limited SRTE (mg l^{-1}): Fe as EDDHA (0.1), B as H_3BO_3 (0.05), Mn as $MnSO_4$ (0.1), Zn as $ZnSO_4$ (0.005), Cu as $CuSO_4$ (0.002), and Mo as $(NH_4)_6Mo_7O_{24}$ (0.001).

2.2.1. Influx kinetics

Michaelis–Menten constants were obtained using the nutrient-depletion procedure described by Claassen and Barber (1976), and modified by Nielsen and Barber (1978). However, instead of monitoring nutrient-depletion of a single plant over time (a temporal gradient), nutrient-depletion was measured over a spatial gradient, similar to when the plants are growing in the CPS. In this flow-through plug-flow reactor system, each plant is adapted to the ion concentration in solution. Four 10.2×366 cm gutters (Genova Products Inc.) with a 5% slope along the long axis were watered with P-limited SRTE described above at a flow rate of 50 ml min^{-1} with a peristaltic pump (model no. 7520-35 Cole Parmer Instrument Co.). Each gutter had 21 plants uniformly spaced 17.4 cm apart. Nutrient concentration was measured at the inlet, between each plant, and at the outlet. The I_{max} (maximum net influx rate), K_m (concentration at one-half I_{max}), and C_{min} (concentration where net influx is zero, the lowest steady-state concentration reached on the depletion curve), were determined.

2.2.2. Diurnal nutrient removal rates

Nutrient removal rates over the day and night cycle was determined with the following procedure. An American Sigma Streamline 800 SL[1] portable autosampler model 1350 (Medina, NY) was set up to sample the outlet of four replicated Genova gutters each containing six plants, having a hydraulic loading rate of 0.8 m^3 m^{-2} per day, at 2 h intervals for 48 h. NO_3-N, P, and K were measured by ion chromatography as describe in Section 2.1.3. Nutrient removal rate per cross-sectional treatment area (length × width) was calculated as:

$$U = L_w(C_{li} - C_{lo}) \tag{1}$$

where U, substrate utilization rate (g m^{-2} per day); L_w, hydraulic loading rate (m^3 m^{-2} per day); C_{li}, concentration of influent (g m^{-3}); C_{lo}, concentration of effluent (g m^{-3}).

2.2.3. Lettuce growth kinetics

All lettuce plants in the six sections of the CPS, plus the group of seedlings that would replace the harvested section in a normal cycle, were harvested at the end of a 4 day growth cycle. Root and shoot dry weights were measured after plants were dried to a constant weight at 65 °C.

2.2.4. Evapotranspiration rate

The rate plants take up nutrients relative to the amount of water transpired determines if the nutrient concentration increases or decreases in the wastewater (Adler, 1998), so water uptake, i.e. transpiration was measured. The average volume of water loss per gram dry matter produced was defined as the evapotranspiration rate. At 1-week-old, seedlings were placed in 4 l containers containing a complete nutrient solution described above. The solution was aerated and topped off to coincide with harvest times. Solution volume was recorded. At study termination, plants were harvested and root and shoot dry weights recorded. The average volume of water loss per gram dry weight produced was determined by dividing the volume of solution added by total dry weight gained over harvest periods. Evapotranspiration rate, in conjunction with the concentration of nutrients in the wastewater, determines whether wastewater can be completely evapotranspired. The evapotranspiration rate was about 0.25 l g^{-1} dry wt. produced; this indicated that wastewater in this study was too dilute by a factor greater than 10 to be completely evapotranspired.

2.2.5. Critical deficiency level

There is a feedback loop relationship that describes the effect of nutrient concentration in plant tissues on nutrient absorption and plant growth. A threshold concentration of a nutrient is necessary to maximize growth (CDL). The CDL is the threshold nutrient concentration in the shoot where the growth rate begins to decrease (5–10% below the maximum), because the specific elemental concentration in the tissue has become too low. To determine the CDL, lettuce seedlings were set in gutters and grown at a mass loading such that the growth rate decreased by the end of the gutter. The CDL was the point where growth decreased.

2.2.6. Set-point concentration

There are genetic constraints on the maximum concentration to which a given nutrient will be absorbed in a plant and a minimum concentration is needed to maintain growth. Plants can absorb some nutrients in excess of their needs, but not above a certain limit. The high nutrient concentration limit is defined as the upper set-point concentration (SPC) (Glass, 1989). The value of the upper SPC was taken as the concentration of the first plant in the gutter where absorption rates were at I_{max} throughout the experiment. As nutrient concentrations decrease from the maximum at the upper SPC to the CDL where the growth rate has begun to decrease, further decreases will lead to the point where plant growth stops. The P concentration where the growth rate is zero (lower SPC) was determined by decreasing the hydraulic loading rate such that most of the plants in the gutter received insufficient P to meet their needs. The concentration at which P stabilized was the concentration where zero growth occurred.

2.3. Parameters and model algorithm

The model that is described below was used to simulate the experimental data collected during operation of the pilot-scale CPS. The model assumptions describing nutrient influx are stated in Table 1. Only plant parameters were included in this model, since the plants were growing in water at high flow velocities, thereby minimizing nutrient gradients and diffusion. Nutrient removal, under these conditions, depends on the mass loading rate, concentration of the specific nutrient in solution, and plant influx kinetics. When the nutrient contents of the lettuce were measured, it was determined that they were within the bounds of the CDL and SPCs, and feedback loops describing the effect of nutrient concentration on plant growth were not needed for the purpose of this study. Consequently, not all parameters that have been measured and defined above were included. Only the subroutines needed to simulate the experimental data collected during operation of the pilot-scale CPS were included in the mechanistic model.

Table 1
Model assumptions

1. Nutrient removal occurs only through plant uptake
2. Nutrient uptake occurs only from nutrients in solution at the root surface
3. The relation between net influx and concentration can be described by Michaelis–Menten kinetics
4. Root growth rate is altered over the continuum from a deficient to luxury nutrient status (e.g. P deficiency increases root growth). However, influx parameters and root growth were measured under steady-state conditions in this experiment and, therefore, the effect of ion concentration on root growth is a part of the empirical data
5. Nutrient influx characteristics are not changed by root age or plant age and, therefore, are assumed not to vary along root length. Although influx kinetics change as roots adapt to low nutrient concentrations, steady-state influx kinetics are assumed. Influx kinetics vary diurnally, however, that change has not been incorporated into this model
6. Phosphorus is the most limiting nutrient
7. The CDL (where growth rate is 5–10% below the maximum) for potassium or phosphorus does not vary with age
8. When the upper SPC is reached, uptake rates are determined by growth rates. Growth ceases when the lower SPC is reached
9. Nutrient diffusion to the root surface was considered negligible due to high water flow velocities

Parameter symbols, definitions, initial values, and units are given in Table 2 for constant and Table 3 for variable parameters. The simulation model was written with STELLA II (High Performance Systems Inc., Hanover, NH), using Euler's method of integration, a step time of 0.0625 days, and a simulation time of 4 days. The model was divided into six replicated subunits, one for each section in the CPS. Each subunit contained the same algorithms for calculating plant nutrient removal from water. The subunits were initialized with values for the constant parameters derived from empirical studies described above. Each of the six sections in the model simulation represents a production area of about 0.637 m^2. Parameters and differential equations used to simulate nutrient and biomass accumulation and water quality in the model are shown below.

2.3.1. Phosphorus and water mass balances

The subunits, i.e. sections in the CPS, are linked by the flow of water and phosphorus. The water flow rate (Q_l^w) and the P concentration (C_l^P) are initial input variables into the model and are subsequently determined as follows. The C_l^P is the product of the mass loading rate of P (Q_l^P) flowing between subunits and the Q_l^w:

$$C_l^P = Q_l^P \times Q_l^w \tag{2}$$

The mass loading rate of P decreases for each sequential subunit as the plants absorb P. The mass P loading rate at the outlet (Q_{lo}^P) of a subunit is calculated from the difference between the subunit inlet P mass loading rate (Q_{li}^P) and the total net P influx rate ($I_n^{P_t}$) across a subunit:

$$Q_{lo}^P = Q_{li}^P - I_n^{P_t} \tag{3}$$

The water flow rate also decreases for each sequential subunit as plants transpire water. The transpiration rate (Q^{R_t}) is directly proportional to the shoot biomass and was expressed on the basis of the shoot growth rate (R_g^s) in the model:

$$Q^{R_t} = R_g^s \times 0.25 \tag{4}$$

The water flow rate for the outlet (Q_{lo}^w) of each subunit is the difference between the inlet water

Table 2
Constant parameter symbols, definitions, initial values, and units

Model parameters	Definition	Value	Units
C_{li}^{K}	Initial K concentration in water	441	μM
C_{li}^{P}	Initial P concentration in water	16.8	μM
Q_{l}^{w}	Water flow rate	0.005	$l\ s^{-1}$
I_{max}^{K}	Maximal net influx of K into roots	52	nmol K g^{-1} root dry wt. s^{-1}
I_{max}^{P}	Maximal net influx of P into roots	4.5	nmol P g^{-1} root dry wt. s^{-1}
C_{min}^{K}	Ion concentration in water at the root's surface where influx = efflux and $I_n^K = 0$	640	nM K
C_{min}^{P}	Ion concentration in water at the root's surface where influx = efflux and $I_n^P = 0$	35	nM P
K_m^K	Michaelis–Menten constant; ion concentration in water, $C_l - C_{min}$, where $I_n = 1/2I_{max}$	20	μM K
K_m^P	Michaelis–Menten constant; ion concentration in water, $C_l - C_{min}$, where $I_n = 1/2I_{max}$	2.2	μM P
Q^{R_t}	Transpiration rate	0.25	$l\ g^{-1}$ root dry wt

Table 3
Variable parameter symbols, definitions, and units

Model parameters	Definition	Units
$I_n^{P_t}$	Total net P influx rate	nmol s^{-1}
I_n^P	Specific P influx per unit mass of root	nmol g^{-1} root dry wt. s^{-1}
Q_{lo}^P	Mass P loading rate	μmol s^{-1}
Q_{li}^P	Subunit inlet P mass loading rate	μmol s^{-1}
Q_{lo}^w	Outlet water flow rate	$l\ s^{-1}$
Q_{li}^w	Inlet water flow rate	$l\ s^{-1}$
m_r	Total root mass	g
Δm_r	Root mass growth	g
R_g^r	Root growth rate	g s^{-1}
m_s	Shoot mass	g
Δm_s	Shoot mass growth	g
R_g^s	Shoot growth rate	g s^{-1}

flow rate (Q_{li}^w) and the Q^{R_t}:

$$Q_{lo}^w = Q_{li}^w - Q^{R_t} \tag{5}$$

2.3.2. Plant growth and phosphorus uptake kinetics

Within each model subunit are subroutines with parameters to describe plant growth and P influx kinetics. The specific P influx per unit mass of root (I_n^P) is described by Michaelis–Menten kinetics; maximum net P influx rate (I_{max}^P), P concentration at one-half $I_{max}(K_m^P)$, and P concentration where net influx is zero (C_{min}^P):

$$I_n^P = \frac{I_{max}^P(C_{li}^P - C_{min}^P)}{K_m^P + C_{li}^P - C_{min}^P} \tag{6}$$

Total net-P influx ($I_n^{P_t}$) is the product of I_n^P and the total root mass (m_r) in a subunit:

$$I_n^{P_t} = I_n^P \times m_r \tag{7}$$

In the Stella program, root mass growth (Δm_r) is modeled based on empirical data showing the change in root mass with time (Fig. 2). The Stella program uses the data provided in Fig. 2 to extrapolate a Δm_r over a given time interval (Δt). The root growth rate (R_g^r) at a given time (t) is then calculated from the change in root mass (Δm_r) during a time interval (Δt) occurring at time t:

$$R_g^r(t) = \frac{\Delta m_r}{\Delta t} = \frac{[m_r(t) - m_r(t - \Delta t)]}{\Delta t} \tag{8}$$

The root growth rate can change when plant nutrient status changes, e.g. root growth increases as P concentrations become limiting in the soil environment. In this experiment, however, influx was measured at steady-state P concentrations, so if low P concentrations increased root growth, they are accounted for in the root data collected.

In this algorithm, shoot mass growth (Δm_s) is modeled based on empirical data showing the change in shoot mass with time (Fig. 2). The Stella

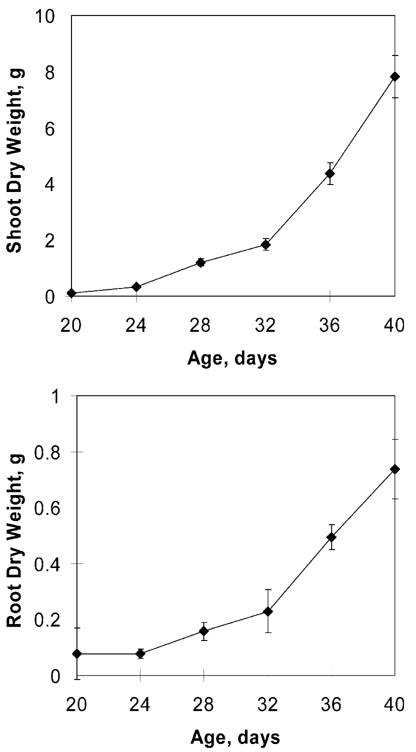

Fig. 2. Each age group represents one of the six sections in the CPS. Shown are the mean root and shoot dry weight for lettuce ($n = 20$) in each section. Vertical bars denote ±S.D.

program extrapolated a Δm_s for a given time interval by extrapolating from the data shown in Fig. 2. The shoot growth rate (R_g^s) at a given time (t) is then calculated from the change in shoot mass (Δm_s) over a short time interval (Δt) occurring at time t:

$$R_g^s(t) = \frac{\Delta m_s}{\Delta t} = \frac{[m_s(t) - m_s(t - \Delta t)]}{\Delta t} \times R_g^{s_c} \quad (9)$$

3. Results and discussion

3.1. Influx kinetics

In addition to P influx kinetics, K influx kinetics were also determined because K is co-limiting with P in aquaculture effluents and the model would allow us to determine how much was needed to prevent it from limiting P uptake. The maximum net influx of P and K into roots was 4.5 and 52 nmol g^{-1} root dry wt. s^{-1}, the Michaelis–Menten constant was 2.2 and 20 μM, and the ion concentration in water where the net influx is 0 was 35 and 640 nM, respectively.

3.2. Diurnal nutrient removal

Wastewater from aquaculture facilities is generated 24 h a day. Unless treatment occurs continuously, effluent would need to be stored. We found that although there is a diurnal pattern of nutrient absorption by lettuce, significant uptake occurs 24 h a day (Fig. 3), a finding reported earlier in a wetland system (Adler et al., 1996a) and observed by others for nitrogen and potassium (Clement et al., 1978; Le Bot and Kirkby, 1992). Only minor diurnal variation, however, occurred with P, the nutrient of focus in this study because of its importance in contributing to eutrophication. Therefore, there is no need to store water during the dark period for treatment during the day and treatment can continue 24 h a day in phase with the fish production system.

3.3. Nutrient removal capacity

Plant growth kinetics, NO_3, P, and K concentration in effluent between each of the sections and

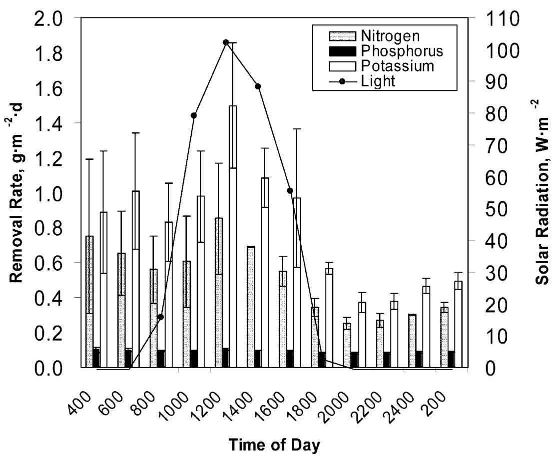

Fig. 3. Nutrient removal rates by lettuce over a 24 h period. Vertical bars denote ±S.D.

within the tissue in each of the sections for lettuce were determined. Growth rates were exponential with about 85% of the growth occurring during the last 12 days of the 40 day production cycle (Fig. 2). Productivity averaged about 12 g m^{-2} per day for lettuce. After steady-state planting and harvesting was achieved, the CPS maintained plant productivity and health while removing 99% of the dissolved phosphorus and 60% of the nitrate from the flow (Fig. 4). Lettuce removed P to less than 1 μg l^{-1}, from an influent concentration of 520 μg l^{-1}. Nitrate could also be removed to these low levels if it was present in balance with the requirements of the other nutrients and not in excess. Removal rates were almost 60 mg P m^{-2} per day with a discharge of less than 1 μg P l^{-1} and about 940 mg N m^{-2} per day with a discharge level of about 5 mg N l^{-1}. Based on an upper SPC of about 0.8% P and a growth rate of about 12 g m^{-2} per day, the maximum P removal rate would be about 100 mg P m^{-2} per day.

In general, if the nutrient being removed was not limiting, removal followed growth, whereas when nutrients were limiting, removal depended on a combination of growth and nutrient concentration in the effluent. If nutrients are not limiting and P concentration in the wastewater is greater than that necessary to achieve I_{max}, 0th order kinetics will describe the relationship and removal will depend on biomass production. Nitrogen removal rates increased as biomass increased, however, for P removal, rates were greatest in sections 4 and 5 for lettuce. Removal was less in the last sections because low concentrations of P in the effluent decreased P uptake kinetics even though plants were larger. Shoot concentrations of N, P, and K were highest in the middle sections where high effluent nutrient concentrations resulted in high influx rates (Fig. 5). As nutrient concentrations decreased, concentrations in plants decreased especially in the case of P, which was the most limiting nutrient. Cellular nutrient concentrations were sufficient to sustain growth even after nutrients within the flow were limiting. The CDL for P in lettuce is between 0.35 and 0.4% and the tissue levels in this experiment never dropped below 0.44% (Fig. 5). This range is similar to what others have found for lettuce (Jones et al., 1991). So we would not have expected to see a reduction in growth rate. This property of P storage through luxury consumption is what allowed the CPS to remove P to such low levels without an apparent reduction in productivity or quality. By positioning plants towards the inlet when they were younger, more nutrients were transferred and stored. These nutrients were available for use when the plants had been moved towards the end of the thin-film gutter and less dissolved nutrients were available.

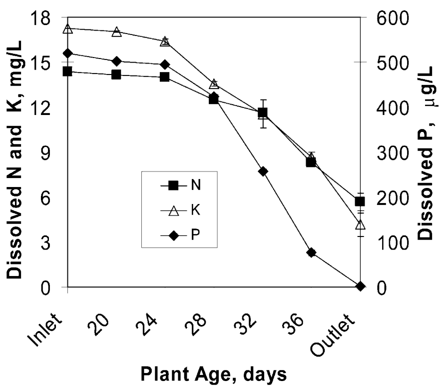

Fig. 4. Mean dissolved nutrient concentrations in the outlet flow from each of the six sections from the CPS where lettuce was grown. Phosphorus was made to be the most limiting nutrient in the effluent so its removal could be maximized. Vertical bars denote ±S.D.

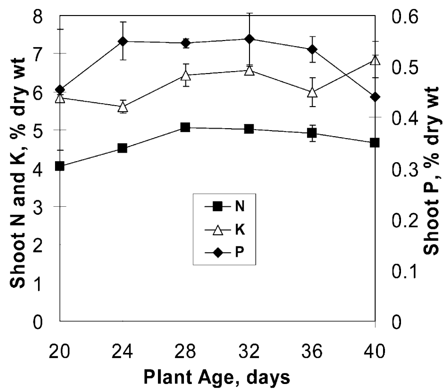

Fig. 5. Mean nutrient concentrations in lettuce shoots from each of the six sections represented by plant age in the CPS. Phosphorus was the limiting nutrient in the effluent and since its concentration in the plant stayed above the CDL, it did not limit growth. Vertical bars denote ±S.D.

The transpiration rate was about 0.25 l water per gram dry weight production. With the high water flow rates through the system, this was only about 2.4% of the total water flow. To transpire all the water, both the nutrients and water need to be balanced with the plants needs (Adler, 1998). In this case, the P concentration in the effluent would need to be about 22 mg l^{-1}.

The SPC were determined for both P and K. The upper SPC for P and K was 0.8 and 8.0%, respectively. At higher effluent concentrations, higher upper SPC values would be expected. The lower SPC for P and K was 0.12 and 0.89%, respectively.

3.4. Treating wastewater with thin-film technology using the CPS

Plants are very effective at removing nutrients to low levels concurrent with the production of a high-value product (Adler et al., 2000a). By using thin-film technology configured in the CPS, this potential was maximized. At sustained solution phosphorus levels >0.3 mg l^{-1}, maximum lettuce growth can be supported (Chen et al., 1997). Influx rates are too low at phosphorus concentrations <0.3 mg l^{-1} to sustain the concentration above the critical level necessary to maintain maximum growth. Plants are able to absorb and store much higher levels of nutrients than is required by their metabolism, a phenomenon called luxury consumption. To maintain high plant productivity, the nutrient influx rate must be high enough so that the nutrient concentration within the plant does not drop below the CDL, that concentration necessary to maintain maximum plant growth. Due to luxury consumption, maximum productivity can be sustained even as nutrients are depleted to C_{min}, the concentration where net influx is zero, if the mean influx rate over the entire plant's growth cycle is high enough to maintain the nutrient concentration above the CDL.

The CPS enables plants to store P early in their growth cycle. This reservoir of P is available for plant growth as P levels in the water decrease and influx can no longer meet current demands. With the CPS, seedlings are introduced at intervals near the inlet of a thin-film system when they are young and sequentially moved progressively towards the outlet where they are then harvested as they mature. At the front end of the thin-film gutters where nutrient concentrations are high, young plants absorb and store nutrients in excess of their immediate needs. Luxury consumption of nutrients during this early growth phase sustains the plants later when they are moved down towards the outlet where nutrient concentrations in solution are too low for absorption kinetics to meet their growth needs.

The nutrient-depletion profile occurs from plant to plant in a plug-flow reactor like the CPS. Therefore, nutrients removed along the entire profile flow to each plant sequentially along the profile, in diminishing amounts. This typically results in reduced productivity at the outlet of plug-flow reactors. By positioning plants towards the inlet when they are younger, more nutrients are transferred and stored, available for future use when less will be available in the solution towards the outlet. The plug-flow reactor design configured in the CPS permits effective nutrient removal, minimizing short-circuiting without an apparent reduction in productivity as P becomes limiting at the outlet.

The number of sections can be greater or less than six. We have not collected data showing the effect of the number of sections on water quality. However, an increase in the number of sections will decrease the percentage of biomass removed with any one harvest resulting in a more stable outlet concentration. The optimum number of sections will depend on the importance of maintaining a stable outlet water quality.

3.5. Experimental simulation

The model was run after being initialized with empirical data and the results of the simulation are graphed along with the empirical data for comparison (Fig. 6). The empirical data reported resulted under an average daily hydraulic loading rate of about 0.113 m^3 m^{-2} per day and average P loading rate of about 59 mg m^{-2} per day. The empirical data describing P depletion in the CPS in Fig. 4 is included in Fig. 6 for comparison with the model simulation of P removal. The model simulation was within about 5% of the empirical data over the range of P concentrations present in this study except at day 36 where the observed data were about three times higher than what was predicted by the model. This indicated that Michaelis–Menten kinetics was generally able to describe P absorption by lettuce except in the range between about 0.75 mg P l^{-1} and C_{min}.

3.6. Economics

The CPS is an effective strategy to remove nutrients in wastewater to very low levels and has previously been evaluated for its economic viability as a water treatment system. Two business scenarios have been evaluated using the CPS to remove nutrients from water: the first compared the CPS as a water treatment system to other standard water treatment technologies (Adler et al., 2000a); the second examined integrating fish and the CPS as a business enterprise (Adler et al., 2000b). The economic analysis indicated that the CPS was competitive with standard water treatment technologies because it generated income while treating wastewater, which can offset treatment costs.

The use of conventional treatment alternatives to remove phosphorus from wastewater, whether they employ chemical precipitation, physical removal, or land application technologies, represent a significant additional cost to the owner of an aquaculture operation. Treatment costs varied from a low of \$0.18 m^{-3} for land application using alfalfa as the recipient crop to a high of \$1.26 m^{-3} for reverse osmosis and electrodialysis (Adler et al., 2000a). They also involve moderate to large investments in capital items that have few alternative uses. In contrast, the CPS generated income while nutrients were removed to a very low level and represented a potentially profitable secondary enterprise for the aquaculture producer.

Integrating fish and plant production resulted in shared cost savings that originate from spreading out operating and capital costs over the two systems. Regardless of the crop chosen (lettuce or basil), expected crop prices appeared to be more than sufficient to cover the costs of production at expected yields. Also, the integration of the two systems reduced the combined consumption of water by reusing water discharged from the fish production system for plant production. Water reuse increased and the majority of this water was returned to the environment in excellent condition. This made the combined systems largely non-consumptive and non-polluting users of the water resource.

The primary drawbacks of hydroponic production as a treatment alternative would be the added technical sophistication, labor, and marketing expertise required. Compared with conventional treatment alternatives that require relatively little additional management or labor, hydroponic production is much more risky. Development of a marketing plan is crucial. Sufficient attention must be paid to the day-to-day operation of the greenhouses and the servicing of markets for the produce or the better profitability of hydroponic production for the treatment of fishery effluent rapidly disappears.

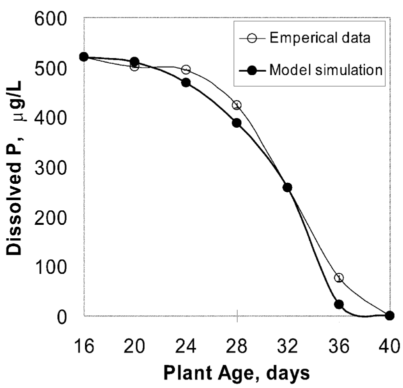

Fig. 6. Comparison of empirical data and the model simulation showing P depletion from RTE in a pilot-scale CPS growing lettuce. Vertical bars on empirical data means denote ±S.D.

4. Conclusions

If all nutrients in the water being treated are equally limiting or balanced, all nutrients can be removed to very low level μg l^{-1} by plants using the CPS. Water with this same nutrient quality can be achieved only by the most advanced water treatment technology, such as ion exchange, reverse osmosis, or electrodialysis. The most elaborate chemical removal systems are expensive and can only remove P to ≈ 0.1 mg l^{-1}. In addition, chemical removal systems generate large amounts of sludge waste. Ion exchange generates a waste with regeneration of the resins, and reverse osmosis and electrodialysis clean a portion of the wastewater with membranes and concentrate ions removed into the waste stream. In contrast, the CPS generates income while nutrients are removed to a very low level. The CPS not only maximizes the P removal potential by plants but also reduces the sensitivity of plants to imbalances of other mobile nutrients in the wastewater. The mechanistic model described in this paper simulated experimental data collected during the operation of the CPS growing lettuce and defines critical data necessary for the general comparison of effluents for treatment and defines critical design parameters needed to engineer these systems.

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